

Pharmacognostic Investigation of Dried Powdered Stem Bark of The Traditional Medicinal Plant *Afzelia Africanus* Used for The Treatment of Haemorrhoids/Piles in Sierra Leone

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Abstract—Pharmacognostic investigation of dried powdered stem bark of the traditional medicinal plant *Afzelia africanus* used for the treatment of Haemorrhoids/Piles in Sierra Leone has been carried out. The results of organoleptic evaluation indicate the powdered stem bark to be light brown in colour with a characteristic wood odour and bitter taste indicating that the plant organ investigated contained alkaloids. The colour of the powdered plant material will also help who so ever wish to buy and use the plant material for medicinal purpose. It helps prevent adulteration. Fluorescence analysis of the plant organ investigated showed that different fluorescent colours were developed when tested with freshly prepared solutions of 1M NaOH (aq), 1M NaOH (alc.), Ammonia, 50% HCl and 50% HNO₃. The result indicates that the plant organ investigated contained crude drugs as it is one of the parameters for pharmacognostic evaluation of crude drugs in traditional medicinal plants. The results phytochemical screening revealed moderate to high contents of carbohydrates, alkaloid, flavonoids, proteins, sterols/terpenes and saponins in the ethanol, methanol and aqueous extracts. All of the solvent extracts apart from the petroleum ether extract revealed high concentration of flavonoids, tannins and phenolic Compounds. The petroleum ether and acetone extracts gave the least concentration of the phytoconstituents investigated. The detection of the above secondary plant metabolites support the use of the plant as food and pharmaceutical in traditional medicine. Elemental analysis was carried out on the plant organ investigated using with a Niton XL3t GOLD + Hand held X-ray Fluorescence (Thermo Fisher). The Niton Hand held XRF Instrument uses aAg-anode X-ray tube with a voltage of 50kV and equipped with a Si-drift detector (SDD). Accurate energy and efficiency calibrations of the spectrometer were made using a certified reference material – SRM 1573a – Tomato Leaves supplied by the International Energy Agency (IAEA), Vienna, Austria. The spectrum acquisition time was 480sec for the sample and the dead time was around 50%. The results of elemental analysis showed that the plant organ

contained large amounts of nutrients and were rich in Ca (42833 ± 251.00 ppm), K (17637 ± 135.00 ppm), Mg (4635 ± 1352 ppm), Al (1868 ± 203.00 ppm), Sc (266 ± 24.00 ppm), and Fe (250.76 ± 10.40 ppm). The other elements present in smaller quantities were Sr (192.55 ± 1.47 ppm), Zn (107.28 ± 3.14 ppm), Ti (106 ± 18.00 ppm), Zr (15.11 ± 14.76 ppm), Rb (14.76 ± 14.76 ppm), Cu (12.07 ± 4.16 ppm) and Mo (6.21 ± 0.76 ppm). The presence of the above elements also support the use of the plant organ investigated in traditional medicine. The elements detected in the plant organ which have both therapeutic and prophylactic properties.

Keywords—Pharmacognostic, haemorrhoids, organoleptic, fluorescence analysis, phytochemical screening, elemental analysis and x-ray fluorescence

INTRODUCTION

This research is geared towards the Pharmacognostic investigation of dried powdered stem bark of the traditional medicinal plant *Afzelia africanus* used for the treatment of Haemorrhoid/Piles in Sierra Leone. The hot aqueous decoction of dried powdered stem barks of the plant is drunk twice a day. In the Southern Province of Sierra Leone the Stem bark is grounded with clay and rubbed on swellings. The heart wood is used to prepare a red dye. The Fruit pod is opened and strapped on the limb to ease "sore" bones. The bark is sometimes used as a Fish poison. The timber is used for making boards and mortars, and the seeds for ornamental purposes. *Afzelia africanus* belongs to the family Caesalpiniaceae with the English name Mahogany and the tree is widely distributed in Africa and Asia [1, 2, 3 and 4].

The leaves of *A. africana* are rich in nitrogen and minerals [4, 5] and have reported to be used as a source of fodder for livestock in the dry seasons. The plant has also been to be widely as folklore remedies among many tribes in Africa [6, 7, 8 and 9].

It has been reported that seeds of *A. africana* are edible, used as soup condiment in Nigeria (rich in oil and used

as thickening agent), as necklaces for ornamental and ritual purposes [7].

The leaves and stem bark extracts of the plant has been reported to exhibit anti-inflammatory and analgesic activities [10], trypanocidal activities [11], treatment for hernia among some tribes in Cote d'Ivoire [12] and as a mouth wash [13].

In Nigeria it has been reported that the whole plant i.e. Roots, Stem bark, leaves and fruits are used in traditional medicine with the root decoctions or macerations used to treat stomach complaints, convulsions, trypanosomiasis and hernia, and as antidote [3]. Root powder used externally to treat rheumatism and to prepare arrow poison. The decoctions of the Stem bark are used for treatment of constipation, fever, vomiting, oedema, tachycardia, hypertension, bronchitis, lung complaints and bleedings during pregnancy, and as anodyne, diuretic, galactagogue and aphrodisiac. The ash obtained from the stem bark is applied externally to treat lumbago, wounds and swellings.

The stem bark is also reported to be used as fish poison, leaf decoctions and macerations for the treatment of dysmenorrhoea, epilepsy, oedema, migraine, stomach-ache, asthenia, trypanosomiasis and as anodyne. Fruit preparations are taken to treat lung complaints and as aphrodisiac. Fruit ash is applied against leprosy and as soap substitute with twigs used as chewing sticks. Antibacterial and antifungal properties of the extracts of the Stem bark of *A. africana* have also been reported [3, 14].

Although various plant parts of *A. africana* are widely used in traditional medicine, few studies on the pharmacognostic investigation have been carried out and hence the purpose of this research works.

Local vernacular names in Sierra Leone

Mende: Kpende
Temne: Ka-KoNTHA
Kono: SeŋGe
Gola: TENA

Trace elements are essential components of biological structures that mediate vital effect on and play a key role in a variety of the biochemical processes necessary for life. Excessive levels higher than that needed for biological functions of these elements can be toxic for

the body health. Hence any Pharmacognostic investigation of traditional medicinal plants without mineral analysis cannot be completed.

MATERIALS AND METHODS

Collection and preparation of dried plant materials

The Stem bark of *A. africana* was collected from the Gola Forest and dried under the shade and not the sun so as to protect the thermo labile components if present from being chemically transformed. It was then reduced in size by crushing it into smaller pieces using the hand with a cutlass. After the plant material had been dried, it was grounded using a laboratory mill and kept in a proper container until the time of the extraction.

The plants organ investigated is the Stem bark with image of the plant shown in Figure 1. A Voucher Specimen No. 402 of dried powdered stem bark of *A. africana* was deposited in the Herbarium of the Botany Department, Fourah Bay College (University of Sierra Leone). The plant material was used to carry out the following analyses described below:

- Organoleptic evaluation
- Fluorescence analysis
- Phytochemical screening
- Mineral analysis

Experimental

Organoleptic characters

Organoleptic evaluation was carried out on the dried powdered stem bark of *A. africana* by means of sense organs, which provide the simplest as well as quickest means to establish the identity and purity of the plant to ensure quality of a particular drug present in it. Organoleptic characters investigated [15] are size, colour, odour, taste and texture of the dried powdered stem bark of *Azelia africana*. The results are shown in Table 1 and the image of powdered plant material in Figure 3.

Fluorescence analysis

0.5mg of dried powdered stem bark of *A. africana* was placed in a glass petri dish free from grease and 2-3 drops freshly prepared reagent solution was added, mixed by gentle with a glass rod and waited for few minutes. The following freshly prepared reagents are used;

1 N NaOH (aq), 1 N NaOH (alc.), Ammonia, Picric acid, Petroleum ether, 50% HCl, 50% H₂SO₄, 50% HNO₃, Ethyl acetate, Ethanol, Methanol, and Bromine water.



Fig. 1: The stem, tree, leaves with fruit and split pods of *Azelia Africana*.

The colours of each of the contents in Petri dish were observed in visible light, short (254 nm) and long (365 nm) ultra violet radiations using a U/V Lamp. A piece of white paper was dipped in each of the solutions and viewed using both visible light and under the U/V Lamp to compare the colours obtained. The colours observed by application of different reagents in different radiations are recorded [16] as shown in Table 2.

Phytochemical analysis

Soxhlet extraction was carried out on the dried powdered stem bark of *A. africana* using solvents of increasing polarity (i.e. Petroleum ether [60-80 ° C], Acetone, Chloroform Methanol, 95% Ethanol and Water. Each of the solvent extracts was concentrated, reduced to a semisolid mass using a Rotary Evaporator at 50°C and kept in special containers for phytochemical screening.

The Phytochemical screening involved testing each of the Solvent Extracts for the various classes of secondary plant metabolites. The methods used for detection of various phytochemicals were followed by qualitative chemical test and by standard procedures [17, 18] to give general idea regarding the nature of constituents present in each of the solvent extracts of the plant part investigated [19, 20, 21, 22, 23, 24 & 25]. They were generally tested for the presence secondary plant metabolites such as Carbohydrates, alkaloids, tannins/phenolic compounds, flavonoids, Sterols/triterpenes, Amino acids/ proteins and saponins/glycosides etc.

Test for carbohydrates

0.5mg of the **Solvent Extract** was dissolved in 5 ml distilled water and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrates.

Molisch's test: -1ml of the extract filtrate was treated with 2 drops of alcoholic α -naphthol solution in a test tube and 1 ml of concentrated tetraoxosulphate (VI) acid was added carefully along the side of the test tube. Formation of violet/purple ring at the junction may indicate the presence of carbohydrate.

Test for reducing sugars

Fehling's test: - 1ml of the extract filtrates was treated with equal volumes of 1ml Fehling A and 1ml Fehling B solutions, boiled for one minute. The mixture was boiled for 5-10 minutes on water bath. The formation of Reddish brown precipitate due to formation of cuprous oxide indicates the presence of reducing sugar.

Benedict's test: - 1ml of the extract filtrate was treated with equal volumes of Benedict's reagent in a test tube. The mixture was boiled for 5-10 minutes on water bath. A change in colour of the solution from blue to green, to yellow or brick-red precipitate depending on amount of test item present indicate the presence of reducing sugar.

Barfoed's Test

1ml of the solvent extract was placed in a boiling tube and 3ml of Barfoed's reagent was added to it. The mixture was heated in boiling water bath for 7 minutes.

Observation

The colour of the solution changes from blue to dirty green to greenish-yellow and then to Dark yellow precipitate with brick-red precipitates seen on top of Dark yellow precipitate indicate the presence of carbohydrate.

Iodine Test:

2-3 drops of iodine solution was added to 1ml of the solvent extracts.

The formation blue-black colour indicates the presence of starch.

Test for Glycosides

Test for cardiac glycosides:

Keller Kelliani test (test for deoxysugar): -The **Solvent Extracts** was treated with chloroform and evaporated it to dryness. 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride was added to it and transferred to a small test tube. 0.5 ml of concentrated tetraoxosulphate (VI) acid was carefully added down the side of the test tube. The formation of a blue colour in the acetic acid layer indicates the presence of glycosides.

Test for Anthraquinone Glycosides

Borntrager's test: - The **Solvent Extracts** was boiled with 1 ml of dilute tetraoxosulphate (VI) acid in a test tube for 5 min and filtered while hot. The filtrate or supernatant layer was pipette out and placed into a test tube. The mixture was cooled and shaken with equal volumes of dichloromethane. The lower levels of dichloromethane was separated and shaken with half its volume with dilute ammonia. The appearance of a rose pink to red colour in the ammonical layer indicates the presence of glycosides.

Test for Saponin Glycosides

Froth test: - The **Solvent Extracts** was treated with water in a small tube and shaken very well. The appearance of a persistent froth on the top of the mixture indicates the presence of glycosides.

Tests for Amino acids and Proteins

Biuret test (General test): - The **Solvent Extracts** was treated with 1 ml 10% sodium hydroxide solution and heated. 2-3 drops of 0.7% copper (II) tetraoxosulphate (VI) solution was added to the mixture stirred and allowed to stand for few minutes. The formation of purplish violet colour may indicate the presence of proteins.

Millions Test (for proteins): -3 ml of the **Solvent Extracts** was mixed with 5 ml Million's reagent separately. The formation of white precipitate which on heating turned to brick red indicated the presence of amino acids.

Xanthoproteic Test: The **Solvent Extracts** was placed in a test tube; 1ml of conc. H_2SO_4 was added to the mixture and boiled for few minutes. 1ml of ammonia solution was added to the mixture. The formation of a white precipitate which on heating turned yellow and orange on addition of ammonia solution indicates the presence of proteins.

Tests for Sterols and Triterpenoids**Libermann-Burchard test**

The Solvent Extracts was treated with few drops of acetic anhydride boiled for few minutes. The mixture was cooled and concentrated tetraoxosulphate (VI) acid added down the side of the test tubes. A brown ring at the junction of two layers with the upper layer turning green indicates the presence of sterols while formation of deep red colour indicates the presence of triterpenoids.

Salkowski's test

Each of the Solvent Extracts was treated with chloroform with few drops of concentrated tetraoxosulphate (VI) acid, shaken well and allowed to stand for ten minutes. The appearance of red colour in the lower layer indicates the presence of sterols while formation of yellow coloured lower layer indicates the presence of triterpenoids.

Tests for tannins and phenolic compounds**Ferric chloride test**

Small amount each of the Solvent Extracts was shaken with water and warmed. 2 ml of 5% ferric chloride solution was added and observed. The formation of green or blue colour indicates the presence of phenols.

Gelatin test

1% gelatin solution containing 10% sodium chloride was added to each of the Solvent Extracts. The formation of white buff coloured precipitate indicates the presence of tannins and phenolic compounds.

Iodine test

Each of the Solvent Extracts was treated with diluted iodine solution. The appearance of transient red colour indicates the presence of tannins and phenolic compounds.

Nitric acid test

Each of the Solvent Extracts was treated with dilute nitric acid separately. The formation of reddish to yellowish colour indicates the presence of tannins and phenolic compounds.

Test for alkaloids

About 0.5mg of each of the Solvent Extracts was stirred with about 5 ml of dilute hydrochloric acid separately and filtered. Each filtrate was tested with the following reagents:

Dragendroff's test

Few drops of Dragendroff's reagent was added to each filtrate and observed. The formation of orange yellow precipitate indicates the presence of alkaloids.

Mayer's test

Few drops of Mayer's reagent (Potassium mercuric iodide solution) was added to the filtrate and observed. The formation of white or cream colour precipitate indicates the presence of alkaloids.

Hager's test

Few drops of Hager's reagent was added to filtrate and observed. The formation of yellow precipitate indicates the presence of alkaloids.

Wagner's test

Few drops of Wagner's reagent (solution of iodine in potassium iodide) was added to filtrate and observed. The formation of reddish brown precipitate indicates the presence of alkaloids.

Tests for flavonoids:**Shinoda's test**

5ml. 95% ethanol was added separately to the Solvent Extracts and the mixture was treated with 0.5g magnesium turnings and few drops of conc. HCl. The formation of pink colour indicates the presence of Flavonoids.

Alkaline reagent test

Lead acetate solution was added to 0.5mg of the Solvent Extracts and observed. The appearance of yellow precipitate after few minutes indicates the presence of Flavonoids.

The same procedure was repeated for all the other solvent soxhlets extracts with results shown in Table 3.

Mineral analysis**Sample preparation**

Sample was thoroughly washed with pure water and rinsed with double distilled water in order to remove the sand or dust particles and all other surface contamination. The plant sample was then air dried, grounded and homogenized in an agate mortar and sieve through a 250µm diameter sieve. A quantity of 3.0g mass of the powdered sample was weighed with an analytical balance and placed in a sample cup holder.

Sample analysis

Elemental analysis of the sample was performed with a Niton XL3t GOLD + Hand held X-ray Fluorescence (Thermo Fisher). The Niton Hand held XRF Instrument uses a Ag-anode X-ray tube with a voltage of 50kV and equipped with a Si-drift detector (SDD). Accurate energy and efficiency calibrations of the spectrometer were made using a certified reference material – SRM 1573a – Tomato Leaves supplied by the International Energy Agency (IAEA), Vienna, Austria. The spectrum acquisition time was 480sec for the sample and the dead time was around 50%.

X-Ray Fluorescence has long been recognized as a powerful technique for the qualitative and quantitative elemental analysis [26, 27]. The equipment is non-destructive, multi-elemental, fast, and cost-effective in determining elements present in a given sample under test. It offered a fairly uniform detection limit across a large portion of the Periodic Table and applicable to a wide range of concentrations.

In this study, a total of fifteen elements (K, Ca, Mg, Al, Ti, V, Mn, Fe, Cu, Zn, Rb, Sr, Zr, Mo, and Sc) were investigated in the dried powdered stem barks of *Azelia africanus* plant by using EDXRF. The mean concentrations of various metals in the plant sample are shown in Table 4.

RESULTS AND DISCUSSIONS

Organoleptic evaluation

The results of organoleptic evaluation of the dried powdered stem barks of *Azelia africanus* plant are reported in Table 1 below with the photo of the dried powdered stem bark of *Azelia africanus* plant shown in Figure 3.

The bitter taste indicates that each of the powdered plant materials contain alkaloids. The colour of the powdered plant material shown in Figure 3 will also help who so ever wish to buy and use the plant material for medicinal purpose. It helps prevent adulteration.

Fluorescence analysis

The results of Fluorescence analysis carried out on the dried powdered stem bark of *Azelia africanus* plant are reported in Table 2.

The table 2 showed a colour changes with the following reagents: 1M NaOH (aq.), 1M NaOH (alc.), Ammonia, 50% HCl, and 50% HNO₃.

Some constituents in plant organs do not show fluorescence in the visible range in daylight. Their derivatives upon reaction of the powdered plant organ with some reagents shown above produce fluorescent activity under UV Light. Fluorescence analysis is one of the parameters for pharmacognostic evaluation of crude drugs [16] in traditional medicinal plants. Thus the process of standardization can only be achieved by stepwise pharmacognostic studies as stated above. This research work helps in identification and authentication of the dried powdered stem bark of *Azelia africanus* plant material used in traditional medicine. Such information can act as reference information for correct identification the dried powdered stem bark of *Azelia africanus* plant and also will be useful in making a monograph of the plant. Further, it will act as a tool to detect adulterants and substituent and will help in

maintaining the quality, reproducibility and efficacy of natural drugs in the plant organ investigated.

Phytochemical Screening

The results of phytochemical screening of the dried powdered stem bark of *Azelia africanus* plant are given in Table 3.



Fig. 2: EDXRF used for elemental analysis of powers plant sample.



Fig. 3: Powered stem bark of Azela africanus.

Table 1: Showing the results of organoleptic evaluation of the dried powdered stem bark of *Azelia africanus* plant.

PLANT ORGAN INVESTIGATED	PROPERTY TESTED				
	COLOUR	ODOUR	TASTE	TEXTURE	PARTICLE SIZE
Stem bark	Light Brown	Wood odour	Bitter	Powdered	100 # wire gauge

Table 2: Results of fluorescence analysis of the dried powdered stem bark of *Azelia africanus* plant.

T est	Powdered plant material	Visible/day light	Ultra violet light
1	Powder	Yellow	Yellow
2	Powder + 1MNaOH(aq)	Yellow	Light orange
3	Powder + 1MNaOH(alc)	Yellow	Bright orange
4	Powder + Ammonia	Light green	Bright orange
5	Powder + Picric acid	Light Yellow	Yellow
6	Powder + Petroleum ether	Yellow	Black
7	Powder + 50% HCl	Yellow	Light blue
8	Powder + 50% H ₂ SO ₄	Yellow	Yellow
9	Powder + 50% HNO ₃	Yellow	Cream white
10	Powder + ethyl acetate	Yellow	Black
11	Powder + Ethanol	Yellow	Black
12	Powder + Methanol	Yellow	Black
13	Powder + Br ₂ water	Yellow	Black

Table 3: Results of phytochemical screenings of the dried powdered stem bark of *Azelia africanus* plant.

Experiment		Solvents					
Secondary Plant Metabolites	Tests/Reagents	PZ	AC	CHLO	MeOH	EtOH	Water
Carbohydrates	Molisch's Test	-	-	-	++	++	+++
	Fehling's Test	-	-	-	++	++	+++
	Benedict's Test	-	-	-	++	++	+++
	Barfoed's Test	-	-	-	++	++	+++
	Iodine Test	-	-	-	++	++	+++
Alkaloids	Mayer's Test	-	-	+	+++	++	+
	Hager's Test	-	-	+	+++	++	+
	Wagner's Test	-	-	+	+++	++	+
	Dragendroff's Test	-	-	+	+++	++	+
Tannins and Phenolic Compounds	Iron(III) Chloride Test	-	++	-	+++	+++	+++
	Gelatin Test	-	++	++	+++	+++	+++
	Iodine Test	-	++	++	+++	+++	+++
	Dil. HNO ₃ Test	-	++	++	+++	+++	+++
Flavonoids	Shinoda's Test	-	+++	++	+++	++	++
	Lead acetate Test	-	+++	++	+++	++	++
	KOH Test	-	+++	++	+++	++	++
Sterols/Triterpenes	Liebermann-Burchard Test	+	-	-	+	+	+
	Salkowski's Test	+	-	-	+	+	+
Amino acids and Proteins	Biuret Test	+	-	+	++	++	+++
	Million's Test	+	-	+	++	++	+++
	Xanthoproteic test	+	-	+	++	++	+++
Glycosides and Saponins	Keller Kelliani Test	-	+	-	+	+	++
	Borntrager's Test	-	+	-	+	+	++
	Froth Test	-	+	-	+	+	++

KEY: PZ = Petroleum ether, AC = Acetone, CHLO = Chloroform, MeOH = Methanol, EtOH = Ethanol; +++ = Intense; ++ = Moderate; + = Slight; - = Absent

Table 4: Showing the total contents of elements (in ppm) in the powdered stem bark *Azelia africanus*

Plant Organ	K	± SD	Ca	± SD	Mg	± SD	Al	± SD
Powdered stem bark	17637	135.00	42833	251.00	4635	1352	1868	203.00
Plant Organ	Ti	± SD	V	± SD	Mn	± SD	Fe	± SD
Powdered stem bark	106	18.00	? LOD	9.80	? LOD	13.16	250.76	10,40
Plant Organ	Cu	± SD	Zn	± SD	Rb	± SD	Sr	± SD
Powdered stem bark	12.07	4.16	107.28	3.14	14.76	0.64	192.55	1.47
Plant Organ	Zr	± SD	Mo	± SD	Sc	± SD		
Powdered stem bark	15.11	0.83	6.21	0.76	266	24.00		

Petroleum ether, acetone, chloroform, methanol, ethanol and aqueous crude extracts of the dried powdered stem barks of *Azelia africanus* plant used for the treatment of Haemorrhoid/Piles in Sierra Leone was evaluated for the presence of secondary plant metabolites.

The Phytochemical evaluation according to Table 3, revealed moderate to high contents of carbohydrates, alkaloid, flavonoids, proteins sterols/terpenes and saponins in the ethanol, methanol and aqueous extracts.

All of the solvent extracts apart from the petroleum ether extract revealed high concentration of flavonoids, tannins and phenolic Compounds. The petroleum ether

and acetone extracts gave the least concentration of the phytoconstituents investigated.

The detection of the above secondary plant metabolites support the use of the plant in traditional medicine.

Mineral analysis

The results of mineral analysis of dried powdered stem bark of *Azelia africanus* plant are reported in Table 4.

The results of the current study as shown in Table 4 revealed that all the metals investigated (K, Ca, Mg, Al, Ti, V, Mn, Fe, Cu, Zn, Rb, Sr, Zr, Mo, and Sc) were accumulated in greater or lesser extent in the powdered

stem bark *Azelia africanus* plant. The plant organ contained large amounts of nutrients and were rich in Ca (42833 ± 251.00 ppm), K (17637 ± 135.00 ppm), Mg (4635 ± 1352 ppm), Al (1868 ± 203.00 ppm), Sc (266 ± 24.00 ppm), and Fe (250.76 ± 10.40 ppm). The other elements present in smaller quantities were Sr (192.55 ± 1.47 ppm), Zn (107.28 ± 3.14 ppm), Ti (106 ± 18.00 ppm), Zr (15.11 ± 14.76 ppm), Rb (14.76 ± 14.76 ppm), Cu (12.07 ± 4.16 ppm) and Mo (6.21 ± 0.76 ppm). The other two elements Mn and V were out of limit of detection of the equipment.

It has been reported that medicinal plants possessed some important elements which have both therapeutic and prophylactic properties [28, 29, 30, 31 and 32]. Excessive levels of these elements in medicinal plants could lead to toxicity. Hence knowledge of the presence and amount of these elements in plants also validates the use of the plant as food and medicine. Fruits and vegetables are safe and valuable sources of minerals [29].

Potassium participates actively in the maintenance of the cardiac rhythm [33] and in constipation. Potassium participates actively in the maintenance of the cardiac rhythm [34] and in constipation. Calcium plays a great role in the prevention or treatment of pre-eclampsia[35], colon cancer [36], or hypertension [37].

Zinc has been reported to be an essential component of a large number (>300) of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. Zinc plays a central role in the immune system, affecting a number of aspects of cellular and hormonal immunity [38].

It has also been reported that severe zinc deficiency in humans causes growth retardation, delayed sexual and bone maturation, skin lesions, diarrhoea, alopecia, impaired appetite, increased susceptibility to infections mediated via defects in the immune system, and the appearance of behavioural changes [39, 40, 41, 42 and 43].

The physiology of iron has been extensively reviewed [44, 45, 46, 47, 48, and 49]. Iron is reported to exhibit several vital functions in the body, a carrier of oxygen to the tissues from the lungs by red blood cell haemoglobin, transport medium for electrons and as an integrated part of important enzyme systems in various body tissues.

Mg has been reported to be a cofactor in formation of more than 300 enzyme systems that regulate diverse biochemical reactions in the body, including protein synthesis, muscle and nerve function, blood glucose control, blood pressure regulation in humans [50, 51], energy production, oxidative phosphorylation, and glycolysis. It contributes to the structural development of bone and is required for the synthesis of DNA, RNA, and the antioxidant glutathione [52]. It protects

mitochondria, which is the storehouse of energy, from the dangerous oxidants [53], transport of calcium and potassium ions across cell membranes, a process that is important to nerve impulse conduction, muscle contraction, and normal heart rhythm [51] and participates actively in the maintenance of the cardiac rhythm [54] and in constipation.

Zn has been reported to contribute to proteins, carbohydrates, lipids metabolism, and energy formation in humans and is vital for the healthy working of many of the body's systems; it plays an essential role in numerous biochemical pathways. It is particularly important for healthy skin production and is essential for a healthy immune system and resistance to infection [55, 56, and 57].

SUMMARY

Organoleptic characters

Organoleptic evaluation comprising the size, colour, odour, taste and texture was carried out on the dried powdered stem bark of *A. Africana*. The powdered stem bark was found to be light brown in colour with a characteristic wood odour and bitter taste indicating that the plant organ investigated contained alkaloids. The colour of the powdered plant material will also help who so ever wish to buy and use the plant material for medicinal purpose. It helps prevent adulteration.

Fluorescence analysis

A portion the dried powdered stem bark of *A. africana* was placed separately in each of glass petri dishes free from grease and 2-3 drops freshly prepared reagent solution of 1 N NaOH (aq), 1N NaOH (alc.), Ammonia, Picric acid, Petroleum ether, 50% HCl, 50% H₂SO₄, 50% HNO₃, Ethyl acetate, Ethanol, ethanol, and Bromine water added, mixed gently with a glass rod and waited for few minutes for the colours to develop. The colours of each of the contents in various Petri dishes were observed in visible light, short (254 nm) and long (365 nm) ultra violet radiations using a U/V Lamp. A piece of white paper was dipped in each of the solutions and viewed using both visible light and under the U/V Lamp to compare the colours obtained.

The results indicated that Some constituents gave fluorescent colour changes in reagents 1M NaOH (aq.), 1M NaOH(alc.), Ammonia, 50% HCl, and 50% HNO₃.

Fluorescence analysis is one of the parameters for pharmacognostic evaluation of crude drugs [16] in traditional medicinal plants.

Phytochemical analysis

Soxhlet extraction was carried out on the dried powdered stem bark of *A. africana* using solvents of increasing polarity (i.e. Petroleum ether [60-80 ° C], Acetone, Chloroform Methanol, 95% Ethanol and Water. Each of the solvent extracts was concentrated, reduced to a semisolid mass using a Rotary Evaporator at 50°C and stored in specialized containers. Phytochemical screening was carried out on the various solvent extracts using standard procedures [17, 18] and qualitative

chemical test to give general idea regarding the nature of constituents present in each of the solvent extracts of the plant part investigated [19, 20, 21, 22, 23, 24 & 25]. The results revealed moderate to high contents of carbohydrates, alkaloid, flavonoids, proteins sterols/terpenes and saponins in the ethanol, methanol and aqueous extracts.

All of the solvent extracts apart from the petroleum ether extract revealed high concentration of flavonoids, tannins and phenolic Compounds. The petroleum ether and acetone extracts gave the least concentration of the phytoconstituents investigated.

The detection of the above secondary plant metabolites support the use of the plant in traditional medicine.

Mineral analysis

Elemental analysis on the dried powdered stem bark of *A. africana* was performed with a Niton XL3t GOLD + Hand held X-ray Fluorescence (Thermo Fisher). The Niton Hand held XRF Instrument uses Ag-anode X-ray tube with a voltage of 50kV and equipped with a Si-drift detector (SDD). Accurate energy and efficiency calibrations of the spectrometer were made using a certified reference material – SRM 1573a – Tomato Leaves supplied by the International Energy Agency (IAEA), Vienna, Austria. The spectrum acquisition time was 480sec for the sample and the dead time was around 50%. A total of fifteen elements (K, Ca, Mg, Al, Ti, V, Mn, Fe, Cu, Zn, Rb, Sr, Zr, Mo, and Sc) were investigated using EDXRF.

The results of the analysis showed that the plant organ contained large amounts of nutrients and were rich in Ca (42833 ± 251.00 ppm), K (17637 ± 135.00 ppm), Mg (4635 ± 1352 ppm), Al (1868 ± 203.00 ppm), Sc (266 ± 24.00 ppm), and Fe (250.76 ± 10.40 ppm). The other elements present in smaller quantities were Sr (192.55 ± 1.47 ppm), Zn (107.28 ± 3.14 ppm), Ti (106 ± 18.00 ppm), Zr (15.11 ± 14.76 ppm), Rb (14.76 ± 14.76 ppm), Cu (12.07 ± 4.16 ppm) and Mo (6.21 ± 0.76 ppm). The other two elements Mn and V were out of limit of detection of the equipment.

The presence of the above elements also support the use of the plant organ investigated in traditional medicine. The elements detected in the plant organ which have both therapeutic and prophylactic properties [28, 29, 30, 31 and 32]. Excessive levels of these elements in medicinal plants could lead to toxicity. Hence knowledge of the presence and amount of these elements in plants also validates the use of the plant as food and medicine. Fruits and vegetables are safe and also other valuable sources of minerals [29].

CONCLUSION

Pharmacognostic investigation involving organoleptic evaluation, fluorescence analysis, phytochemical analysis and mineral analysis was carried out on the dried powdered stem bark of the traditional medicinal plant *Azelia africanus* used for the treatment of Haemorrhoids/Piles in Sierra Leone. The results of

organoleptic evaluation indicate the powdered stem bark was found to be light brown in colour with a characteristic wood odour and bitter taste indicating that the plant organ investigated contained alkaloids. The colour of the powdered plant material will also help who so ever wish to buy and use the plant material for medicinal purpose. It helps prevent adulteration.

Fluorescence analysis of the plant organ investigated showed that different fluorescent colours were developed when tested with freshly prepared solutions of 1M NaOH (aq), 1M NaOH (alc.), Ammonia, 50% HCl and 50% HNO₃. This result indicates that the plant organ investigated contained crude drugs as it is one of the parameters for pharmacognostic evaluation of crude drugs [16] in traditional medicinal plants.

The results phytochemical screening revealed moderate to high contents of carbohydrates, alkaloid, flavonoids, proteins, sterols/terpenes and saponins in the ethanol, methanol and aqueous extracts. All of the solvent extracts apart from the petroleum ether extract revealed high concentration of flavonoids, tannins and phenolic Compounds. The petroleum ether and acetone extracts gave the least concentration of the phytoconstituents investigated.

The detection of the above secondary plant metabolites support the use of the plant as food and pharmaceutical in traditional medicine.

The results of elemental analysis showed that the plant organ contained large amounts of nutrients and were rich in Ca (42833 ± 251.00 ppm), K (17637 ± 135.00 ppm), Mg (4635 ± 1352 ppm), Al (1868 ± 203.00 ppm), Sc (266 ± 24.00 ppm), and Fe (250.76 ± 10.40 ppm). The other elements present in smaller quantities were Sr (192.55 ± 1.47 ppm), Zn (107.28 ± 3.14 ppm), Ti (106 ± 18.00 ppm), Zr (15.11 ± 14.76 ppm), Rb (14.76 ± 14.76 ppm), Cu (12.07 ± 4.16 ppm) and Mo (6.21 ± 0.76 ppm).

The presence of the above elements also support the use of the plant organ investigated in traditional medicine. The elements detected in the plant organ have both therapeutic and prophylactic properties.

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